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Ascites and venous carbon dioxide tensions in juvenile chickens of highly selected genotypes and native strains

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A previous study by this group demonstrated that a high carbon dioxide tension in venous blood ($pvCO_2$) of juvenile broiler chickens is a reliable predictor for ascites susceptibility.
In a new experiment with five highly selected genetic stocks and two ascites resistant old breeds we studied levels and variability of $pvCO_2$ within each stock at an early age. Effects of different selection traits (principally growth rate) between fast growing sire lines and slower growing dam lines and a commercial hybrid on blood gas (pCO_2, pO_2) tensions, pH and haematocrit in venous and arterial blood were examined at different ages and compared to values found in ascites resistant breeds. All birds were housed in floor pens in a climate controlled room and subjected to an ascites-predisposing cold environment.
From each stock, 16 birds with the highest (high risk: HRc) and 16 birds with the lowest (low risk: LRc) $pvCO_2$ values were selected at 12 days of age. These birds were marked for future blood sampling to determine changes in haematological characteristics with age and to relate these values to ascites susceptibility. At day 14, eight non-selected birds from each stock were randomly chosen for dissection to determine initial pulmonary arterial pressure index (API) values. Subsequently, all birds were allotted to 8 floor pens (13 birds per pen including two HRc and two LRc birds) per stock. Production performances from 104 birds per stock were measured from 16 to 33 days of age (feed intake (FI); feed conversion ratio (FCR); body weight (BW) at day 33). Mortality was recorded during the complete experimental period. At 5 wk of age, all HRc and LRc birds were necropsied and API values were recorded, which was used to classify the severity of the ascites syndrome.
A convincing effect of $pvCO_2$ values in juvenile chickens on API at 5 wk of age in modern lines confirmed results obtained in the previous study. At an early age, pvO_2 values were much less predictive for high pulmonary pressure induced ascites at wk 5 than $pvCO_2$ values. Hypercapnia combined with low blood pH values and followed by hypoxemia (inducing high haematocrit values) provoked a marked high incidence of ascites and high API values in modern breeds.

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A total absence of ascitic symptoms within native breeds corresponded with unchanged low API values during ageing from 12 to 33 days of age and with lower pCO_2 values in venous and arterial blood compared to modern breeds at all ages. The $pvCO_2$ difference (mean values) between HRc and LRc groups were similar for all modern lines irrespective of age and showed no relationship to growth rate. API, as a reliable indicator for ascites susceptibility, of modern breed chickens correlated with $pvCO_2$ values, but not with growth rate. The high correlation between $pvCO_2$ in juvenile chickens and API values at 5 wk of age indicated that a strong genetic selection pressure on low $pvCO_2$ values at an early age will be an effective method to reduce decisively the occurrence of the ascites syndrome at sea level.

Keywords: ascites; broiler chickens; genetic stocks; blood gas tensions; predicting susceptibility; haematological characteristics

Introduction

Until now there is no consensus about the origin of the ascites syndrome in broiler chickens at sea level. At high altitudes, chronic hypoxia (a reduced oxygen pressure in the air) induces a marked increase in pulmonary arterial pressure and right ventricular hypertrophy in chickens followed by ascites (Sillau *et al.*, 1980). Powell (2000) suggested that the increased growth rate in modern fast growing broiler chickens at sea level requires a higher metabolic rate and thus an increased oxygen supply, which stimulates cardiac output. According to Powell, the chronic increase in cardiac output causes pulmonary hypertension and ascites.

Hypoxia, followed by hypoxemia (a reduced partial oxygen pressure in venous blood) exerts vasoconstriction in the lung of mammals and birds, which causes increased pulmonary arterial pressure and a higher workload of the right ventricle (Marshall and Marshall, 1983; Holle *et al.*, 1978; Scheid and Holle, 1978). This response of vasoconstriction is accounted for by smooth muscle cells in the pulmonary arterial wall responding to the O_2 tension in its vicinity (Marshall and Marshall, 1983). Endothelin, which is produced by endothelial vascular cells during hypoxia is one of the most potent and long lasting vasoconstrictors known (Rakugi *et al.*, 1990); a spectacular dose-response relationship of the vasoconstriction effect of endothelin was found on human pulmonary artery branches (Yanagisawa *et al.*, 1988). As a result of the arterial vasoconstriction in main regions of pulmonary vasculature recruitment of all capillaries occurs, which means a redistribution of blood from the bottom to the top of the lung facilitating gaseous exchange. However, this implies a considerable increase in blood flow resistance resulting in a higher work load of the right ventricle of the heart (Scheele, 1996).

In addition to the vasoconstriction effect, another effect of high altitude hypoxia on pulmonary arterial pressure is tachycardia (an increased frequency of heart muscle contractions) Bouverot (1985). Hypoxemia forces the heart to a higher activity that might result in a higher cardiac output to meet the oxygen requirements of tissues. In acute hypoxia, the increase in cardiac blood flow may contribute to the delivery of oxygen to the various tissues (Nesarajah *et al.*,1983). Sillau *et al.* (1981) demonstrated that increasing the blood flow through the right lung in chickens (caused by an artificial occlusion of the left pulmonary artery), produces right ventricle hypertrophy. Moreover at high altitudes, hypoxemia provokes hyperventilation followed by hypocapnia (a low pCO_2) and alkalosis (Bouverot, 1985).

According to Powell (2000), effects of a fast growth rate in modern broiler lines are comparable to effects of hypoxia at a high altitude. However, observations in experiments with chickens at sea level have shown that neither effects of a fast growth rate nor some typical symptoms of the ascites syndrome at sea level are comparable to the effects of high altitude hypoxia as described by Bouverot (1985) and Sillau (1981):

First, Kuenzel and Kuenzel (1977) showed that an increased growth rate of chickens by genetic selection, coincided with a lower metabolic rate (a decreased oxygen consumption per metabolic weight), which in turn correlates with a decreased heart rate (Van Kampen *et al.*, 1978). Malan *et al.* (2003) found that fast growing broiler chickens compared to slow growing populations exhibited distinct lower metabolic rates. These fast growing birds produced less heat and consumed less oxygen per metabolic weight. Therefore, a fast growth *per se*, inducing a lower heat production and oxygen consumption per metabolic weight does not have to be associated with tachycardia or with a high cardiac output.

Second, Barbato (1997) concluded that an increased genetic emphasis on the early exponential phase of broiler growth appears to result in a healthier chicken. They showed that a high early growth rate *per se*, is not genetically related to any of the deleterious phenotypes showing a high ascites incidence.

Third, Olkowski and Classen (1998) found that ascites susceptibility of fast growing broiler chickens at sea level was associated with bradycardia, which is the opposite of tachycardia, observed in chickens at high altitudes. Experimental results of Olkowski *et al.* (1999) highlighted that a low cardiac output is the haemodynamic key problem leading to cardiovascular failure in fast growing broilers.

Finally, hypocapnia as the result of a hyperventilatory responsiveness to hypoxemia is an unknown factor in fast growing ascitic chickens at sea level. On the contrary, different experiments at sea level showed that hypercapnia is an evident feature in ascites susceptible broiler populations (Buys *et al.* 1999; Olkowski *et al.* 1999; Scheele *et al.* 2003a). These experiments confirmed results from Reeves *et al.* (1991) who discovered hypoventilation as an important feature in ascites susceptible chickens at sea level.

Balog (2003) noticed that in the past, a lot of the blame for our current ascites situation has been placed on genetic selection for increased production performances (*e.g.* increased growth rate). Experimental results of Balog *et al.* (2001) showed that genetic selection for ascites resistance or susceptibility did not affect weight gain, clearly indicating that there is no relationship between growth rate and ascites susceptibility.

Blaming genetic selection for rapid growth, as if this be responsible for a stimulated cardiac output causing pulmonary arterial hypertension, appears to be incorrect by lack of circumstantial evidence. Peacock *et al.* (1989), comparing differences in growth rate within one population of chickens, found that a reduced growth rate by restriction of feed intake prevented the development of right ventricular hypertrophy in response to hypoxia. Olkowski and Classen (1998) showed that heart rates in feed restricted broilers were clearly higher than in *ad libitum* fed birds. A low heart rate and a reduced blood flow in *ad libitum* fed birds may lead to an insufficient supply of oxygen and therefore to hypoxemia and subsequently to pulmonary vasoconstriction and right ventricle hypertrophy. Currie (1999) advocated that a moderate tachycardia induced by feed restriction may protect the bird against those pathological consequences of a low heart rate, by raising its cardiac output. Moreover, a reduced growth rate of fast growing chickens induced by a low protein diet enhances triiodothyronine (T3) levels in blood (Buyse *et al.* 1992, 1994). High plasma T3 levels stimulate the production of free energy (increased heat production) and organ activity, especially the heart. These experiments do not substantiate growth rate as a central factor in ascites aetiology.

In addition to cardiac insufficiency, the occurrence of ascites in chickens at sea level also can originate with a malfunction of the respiratory system causing disturbances in

blood gas tensions. A study by Wideman *et al.* (1999) in broilers showed that a moderate increase in CO_2 concentrations of arterial blood (up to 55 mm Hg) in concert with a decreased pH, by inhalation of 5% CO_2, clearly increased pulmonary arterial pressure. Birds with those increased arterial CO_2 values are apparently predisposed to ascites. Reeves *et al.* (1991) first recognized that at sea level other factors might be responsible for the ascites syndrome in chickens than were known at high altitudes. They found in ascites sensitive chickens that restriction of feed was associated with a marked improvement in variables relating to ventilation such as tidal volume. These authors called attention to the phenomenon that susceptible chickens have impaired ventilatory drives and suffer from relative hypoventilation at an early age, which may cause an elevated partial pressure of CO_2 in blood. Although direct evidence is absent showing that ascites is caused by genetic selection for increased production performances, there is little doubt that different genetic components are related to haematological and cardiovascular disorders leading to ascites (Barbato, 1997; Decuypere *et al.*, 2000; Wideman, 2000; Balog, 2003). These genetic components may have relationships to some aspects of production performances. Scheele *et al.* (2003a) suggested that a high feed efficiency (*i.e.* a reduced heat production) can be realised in selection programmes by an unintended selection for a reduced physical activity, induced by an impaired respiratory activity. This causes a decreased supply of oxygen as well as high pCO_2 values. They found in two fast growing broiler breeds a striking variability in $pvCO_2$ values: markedly high $pvCO_2$ values in venous blood at 2 wk of age predicted high values for pulmonary arterial pressure index (weight of right ventricle divided by weight of both ventricles + septum) at wk 5.

The current experiment was carried out to validate this observation using different broiler populations and to obtain further insight into effects of differences in growth rate on susceptibility for ascites. In this experiment with five highly selected genetic stocks, we examined the effect of differences in production performances between fast growing sire lines and slower growing dam lines and a commercial hybrid on variability of $pvCO_2$ values within each stock at an early age. These effects in the five modern broiler breeds were compared to two ascites resistant old breeds, as to susceptibility for ascites (by measuring pulmonary arterial pressure index) and as to $pvCO_2$ values at day 12. Another objective of the present study was to assess the effects of all stocks and of $pvCO_2$ at day 12 (as predictor for ascites susceptibility), on following haematological characteristics: pvO_2, $pvCO_2$, haematocrit, pH in venous blood at different ages, and $paCO_2$, paO_2, haematocrit, and pH in arterial blood and on pulmonary arterial pressure index, at 5 wk of age. Based on these data we substantiated the role of haematological characteristics to the development of the ascites syndrome in chickens.

Materials and methods

CHICKENS

Descriptions of the genetic stocks, husbandry procedures, and temperature regime are described in detail by Malan *et al.* (2003) who published part of the data from this experiment. Chickens of seven different genetic stocks were used. The chicken genotypes, obtained from different breeder companies, were respectively: two pure broiler breeder sires (S1 and S2); two pure breeder dams (D1 and D2); a fast growing commercially available broiler cross (Ross, BC); two ascites resistant old breeds, normally kept under extensive conditions, one originated from France, Label Rouge (LR) and one from Belgium, Mechelse Koekoek (MK). In dam lines, generally, selection pressures on growth rate and feed conversion ratio are much more moderate than in sire lines as dam lines are additionally selected for egg production.

PRE-EXPERIMENTAL PERIOD

Housing and feeding

126 day-old male chickens of each stock were wing-banded for individual identification and housed on wood shavings until 16 days of age. The experimental room was climate-controlled. In order to increase the development of ascitic symptoms, the birds were subjected to a mildly challenging temperature regimen. At one day of age ambient temperature (Ta) was 33ºC, which was reduced after the first day by 3ºC and subsequently once a day by 1ºC, until a temperature of 15ºC was reached at 16 days of age. Subsequently, the ambient temperature was kept constant. Continuous light was given during the first two days of age, followed by a light regimen of 1h darkness and 23 h of light.

A commercial broiler starter diet was fed for *ad libitum* intake until 16 days of age. Dietary nutrient levels were according to normal Dutch practise (CVB, 2001 and NRC, 1994).

Selection of chickens on differences in carbon dioxide tensions in venous blood

On day 12, venous blood samples were collected from the wing vein by venepuncture from 504 birds (72 birds, randomly chosen, of each stock) in total. All samples were immediately analysed for venous oxygen (pvO_2) and carbon dioxide ($pvCO_2$) tension and pH value (blood gas analyser, ABL 605; Radiometer systems, Copenhagen, Denmark). Haematocrit values were measured in all samples as a volume percentage after centrifugation of the blood. Based on blood gas measurements, 16 birds with the highest $pvCO_2$ values (high risk: HRc) and 16 birds with the lowest $pvCO_2$ values (low risk: LRc) were selected per broiler stock and additionally marked by two colours. On day 14, all birds were weighed individually. At the same day, eight non-selected birds from each stock were randomly chosen and killed by carbon dioxide inhalation. Hearts were removed, weighed and dissected out for determination of the initial pulmonary arterial pressure index (API) values, which were calculated as weight of right ventricle divided by weight of both ventricles + septum, and expressed as a percentage. On day 15, eight groups of 13 chickens (incl. two HRc and two LRc birds) per broiler stock were formed (in total 56 groups). Within each stock, the eight groups had approximately the same mean body weight. On day 16, all 56 groups were randomly allotted to 56 floor pens. The remaining birds were removed from the experiment.

EXPERIMENTAL PERIOD AND OBSERVATIONS

The experimental period lasted from 16 to 35 days of age. The birds were fed *ad libitum* a broiler grower diet formulated according to the Dutch requirement tables (CVB, 2001) and NRC (1994). Feed intake and body weight (BW) per pen were measured weekly. At 3 and 4 wk of age, blood samples were collected from the pre-selected HRc and LRc birds per cage, from the wing vein by venepuncture. At 5 wk of age, all selected birds were anaesthetised by intravenous injection of Propofol[1] (0.5 mg/kg body weight) into the wing vein. Subsequently, the chest cavity was opened and arterial blood samples were taken from the left heart ventricle. Heart and liver were removed and weighed. The following ascites related symptoms were visually assessed: the presence of accumulated fluid in the abdominal cavity (ascites), hydropericardium, heart dilatation, and liver cirrhosis. The observation of hydropericardium and of heart dilatation was classified as heart failure syndrome (HFS). Hearts were dissected out for determination of API values according the method as mentioned before. Venous and arterial blood samples were immediately analysed for pCO_2, pO_2, pH, and haematocrit values. All blood sampling and organ collection procedures were carried out according to the regulation of the Animal Care and

Ethics Committee of ID–Lelystad BV. Mortality was recorded daily during the experimental period. On all dead birds the previously mentioned ascites related symptoms were assessed. The total number of recorded symptoms per stock was obtained by adding all observations on dead birds including those in sacrificed selected birds at 5 wk of age.

STATISTICAL ANALYSIS

The data were analysed by ANOVA (Genstat 5, release 4.1), using stock and $pvCO_2$-risk as treatment factors. Least significant differences based on two sided tests ($\alpha = 0.05$) were calculated according Satterthwaite (1946).

Results

PRODUCTION CHARACTERISTICS AND INCIDENCES OF ASCITIC SYMPTOMS

Chicken's BW at 33 days of age, and FCR in the period from 16 to 33 days of age are given per stock in *Table 1*. Production performances of the five modern broiler lines reflected the different goals in selection programmes per stock. Sire lines (S1 and S2) showed fast growth rates combined with low FCR values. Dam lines partly selected for egg production exhibited less favourable production performances compared to sire lines. The commercial broiler cross (BC) inherited high broiler performance values from sire lines. Striking differences in production performances and in ascites incidences were found between modern lines and old breeds. Slow growing LR and MK chickens showed high FCR values compared to modern broiler chickens. The observed numbers of birds with incidences of ascites in the period from 2 to 5 wk of age is given as percentages of the initial number of birds at 2 wk of age. Stimulated by a low ambient temperature, sire lines showed a high incidence of ascites. Dam lines appeared to be significantly ($P<0.001$) less susceptible for the ascites syndrome than sire lines, but still approximately 5% of these birds demonstrated distinctive signs of metabolic disorders. In case of ascites susceptibility, fast growing BC birds now displayed the same picture as dam lines. No single case of ascites or heart failure syndrome, or liver cirrhosis was found in old native strains reared in a cold environment. Mortality of LR and MK birds in the experimental period was less than 0.5% and was not related to ascites.

At 2 wk of age, all stocks showed comparable average API values of approximately 20% (*Table 1*).

VALUES FOR $PVCO_2$, PH AND PULMONARY ARTERIAL PRESSURE INDEX AT DIFFERENT AGES

All stocks, including ascites resistant old breeds, displayed a notable variability in $pvCO_2$ at 12 days of age. Therefore, it was possible to select within each stock two risk-groups of 16 birds exhibiting distinct and significantly different mean values of $pvCO_2$ (differing at least by 7 mm Hg). $pvCO_2$ ranges in old breeds were distinctly smaller than those in modern broiler lines (the smallest range (34 to 45 mm Hg) was shown in LR birds and the largest range (36 to 73 mm Hg) in S2 birds). The probability values of significant effects of stock and pCO_2 risk factor and their interactions on pCO_2, pO_2, pH, and haematocrit values in venous blood at different ages and on pulmonary arterial pressure index at wk 5 are given in *Table 2*. As to haematological values at day 12, all mean values per combination of experimental factors are shown in *Tables 3 and 4* as well as all significant ($P<0.05$) interaction values obtained in wk 3, 4, and 5. By absence of interaction effects for pH at wk 3 and 4 and for haematocrit at wk 4, only main effects per treatment factor are given in *Table 5*.

A prominent interaction effect of stock x $pvCO_2$-risk on $pvCO_2$ values at different ages

accentuated that effects of stock on $pvCO_2$ values were different for both risk groups. Mean $pvCO_2$ values of high risk (HRc) groups of S1, S2, D2, and BC at day 12, were noticeably higher than of D1, LR, and MK populations. Mean $pvCO_2$ values of low risk (LRc) groups of S1, D1, BC, LR and MK birds at day 12, were rather similar, whereas values of S2 and D2 birds were clearly higher. At 3 and 4 wk of age this picture had been changed, as now comparable $pvCO_2$ values of high risk groups of all modern stocks (S1, S2, D1, D2, BC) were markedly increased during ageing in contrast to a relatively small or no increase of $pvCO_2$ in old breeds (LR, MK). Also $pvCO_2$ values of low risk groups of all modern lines increased manifestly during ageing, whereas $pvCO_2$ of low risk groups of LR and MK birds increased moderately. Consequently, at 4 wk of age, so-called low risk groups of all modern breeds exhibited higher $pvCO_2$ values than high risk groups of LR and MK birds at the same age.

Values for pH at day 12 differed highly significant ($P<0.001$) within and between stocks. Highly significant ($P<0.001$) differences in pH values between stocks and between $pvCO_2$-risk groups in venous blood at 3 and 4 wk reflected differences in $pvCO_2$ values (*Table 5*). Relatively low pH values in all modern breeds coincided with high $pvCO_2$ values, and higher pH values in LR and MK chickens corresponded with low $pvCO_2$ values. Significant ($P<0.001$) differences in $pvCO_2$ values between both risk groups were matched by significant ($P<0.001$) differences in pH values.

In this study the pulmonary arterial pressure index (API) was used to classify the severity of the ascites syndrome in chickens at 5 wk of age as is shown in *Table 3*. API values of ascites resistant breeds (LR and MK) at wk 5 were the same as shown at day 12 (*Table 1*). No effects of $pvCO_2$-risk groups on API were found in these birds. Complete different observations were obtained in modern broiler breeds. First, API values of these birds (except low risk BC birds) at wk 5 were distinctly higher compared to 12 days of age. Then high risk groups of S1, S2, D2, and BC birds, exhibiting at day 12 mean $pvCO_2$ values higher than 50 mm Hg, showed at wk 5 significantly ($P<0.05$) higher API values than low risk groups of the same populations. This result illuminated that noticeably high $pvCO_2$ values at an early age can predict the occurrence of ascitic signs during ageing. API values of high risk groups of modern breeds were also notably higher than API values of LR and MK birds. High risk groups of D1 with a mean $pvCO_2$ value of 47.9 mm Hg at day 12 did not show increased API values at wk 5 compared to low risk D1 birds, but also these API values were significantly ($P<0.05$) higher than in ascites resistant LR and MK chickens. Except for low risk BC birds, all so-called low risk groups of modern lines showed startling higher API values at 5 wk of age than shown by ascites resistant breeds.

VALUES FOR PVO_2, AND HAEMATOCRIT AT DIFFERENT AGES

At day 12 we found highly significant ($P<0.001$) main effects of stocks and $pvCO_2$-risk on pvO_2 values. As to stocks: Low pvO_2 values were shown in S1, S2, and D2 populations. Relatively to those chickens, high pvO_2 values were found in D1, BC, LR and MK birds. As to $pvCO_2$-risk: Differences in pvO_2 values between risk groups were only shown in four populations (S1, S2, D1, and D2). A significant ($P<0.05$) and highly significant ($P<0.001$) interaction effect of both experimental factors on pvO_2 at wk 3 and at wk 4 elucidated that $pvCO_2$-risk primarily affected pvO_2 significantly only in a few stocks.

In general, no clear relationships between $pvCO_2$ and pvO_2 values were found. Important differences in $pvCO_2$ between high and low risk groups of BC, LR, and MK flocks at day 12 coincided with no or negligible differences in pvO_2. Within populations of S1, S2, D1, and D2 differences in pvO_2 between high and low risk groups were much smaller than differences in $pvCO_2$. At wk 3, especially populations S1 and D1 exhibited a marked and highly significant ($P<0.001$) difference in $pvCO_2$ between both $pvCO_2$-risk groups, while no significant difference in pvO_2 values was found due to $pvCO_2$-risk in S1

and D1. At wk 4 of age significant differences in $pvCO_2$ between $pvCO_2$-risk groups within D1, BC, LR and MK populations were not accompanied by significant differences in pvO_2 values. Pronounced differences between stocks in effects of $pvCO_2$-risk on pvO_2 resulted in a highly significant ($P<0.001$) interaction effect on pvO_2. No effect of $pvCO_2$-risk on pvO_2 values was found in LR and MK chickens irrespective of age.

On the other hand, particularly high $pvCO_2$ values in the HRc groups of S1, S2 and D2 chickens at wk 4, which went along with exceptionally high API values at wk 5, now coincided with apparently low pvO_2 values at wk 4. In these birds hypoxemia may have contributed to an increased pressure in pulmonary arteries, and to incidences of ascites. Comparing different stocks, we observed that within all modern stocks pvO_2 values decreased markedly during ageing, whereas in LR and MK birds pvO_2 values remained constant from day 12 until 4 wk of age. Compared to LR and MK birds, LRc BC birds demonstrated similar pvO_2 values at day 12 and comparable API values at 5 wk of age, but still in these birds pronounced low pvO_2 values were found at wk 4. The latter observation suggests that a development of hypoxemia *per se* is not necessarily responsible for an increased pulmonary arterial blood pressure. High $pvCO_2$ values at day 12 and a combination of high $pvCO_2$ and low pvO_2 values at wk 4 in high $pvCO_2$-risk BC birds generated significantly ($P< 0.05$) higher API values than were found in ascites resistant breeds LR and MK.

Haematocrit values at day 12 were except for D2 (high values), similar for all stocks. Noteworthy ascites sensitive sire lines S1 and S2 exhibited similar or even lower haematocrit values than ascites resistant breeds LR and MK at this age. Only two stocks (S1 and D2) showed a significant ($P<0.001$) effect of risk groups on haematocrit. In these birds, a high $pvCO_2$ coincided with increased haematocrit values. In all other stocks no significant ($P<0.05$) differences in haematocrit between risk groups were shown at day 12. Analogous to ageing, effects on $pvCO_2$, on pvO_2 and on API values, haematocrit values changed in ageing modern breeds, but remained practically constant in LR and MK birds. Haematocrit of HRc groups of the populations D1, D2, and BC increased faster during ageing than compared to LRc groups. The result was a highly significant ($P<0.001$) effect of $pvCO_2$-risk on haematocrit at 3 and 4 wk of age. The interaction effect of stock x $pvCO_2$-risk on haematocrit at wk 3 is merely due to exceedingly high haematocrit values in high $pvCO_2$-risk groups of the D2 population.

HAEMATOLOGICAL CHARACTERISTICS IN ARTERIAL BLOOD AT WK 5

Probability values of significant effects of stock and risk factor on blood gas tensions, pH, haematocrit values in arterial blood are given in *Table 6*. Mean values of these variables calculated per main factor are presented in *Table 7*.

As to stocks: The results in *Tables 6 and 7* show an evident effect of different groups of populations on arterial blood gas tensions. Notably high values for $paCO_2$ and low values for paO_2 values at wk 5 were found in both sire lines (S1 and S2). Dam line (D2) and broiler cross (BC) showed discernibly less abnormal arterial blood gas tensions than S1 and S2 birds, but still in D2 and BC birds remarkably high $paCO_2$ and low paO_2 values were found. Next, dam line D1 showed significantly ($P< 0.001$) lower $paCO_2$ and higher paO_2 than former lines. Less extreme arterial blood gas values in D2, BC, and D1 birds compared to S1 and S2 birds is in accordance with a significantly ($P<0.001$) lower susceptibility of D2, BC and D1 for ascites than S1 and S2 chickens (*Table 1*). The ascites resistant breeds LR and MK generated manifestly the lowest $paCO_2$ and the highest paO_2 values.

As to the effect of $pvCO_2$-risk: *Tables 6 and 7* show a highly significant ($P<0.001$) effect of $pvCO_2$-risk at 2 wk of age, on $paCO_2$ and no effect on paO_2 at wk 5. The effect of stock on pH values, at wk 5 was completely caused by peculiarly low pH values in both sire

lines (S1 and S2) compared to all other populations. Similar pH values were found in old breeds (LR and MK) and in populations D1, D2 and BC. Obviously meaningful differences in $paCO_2$ values between old breeds and D1, D2 and BC were not accompanied by different pH values. At wk 5 values for pH in arterial blood of those lines were completely not affected by $pvCO_2$-risk; this suggests buffering actions to alleviate negative effects of a modest augmentation of $paCO_2$.

Haematocrit values were significantly ($P<0.001$) affected by stock. The variability of haematocrit values between stocks displayed, in reverse, basically the same picture as was shown for paO_2 values at the same age. High haematocrit values in S1, S2, D2, and BC lines coincided with low paO_2 values, and relatively low haematocrit values in stocks LR, MK, and D1 were consistent with relatively high pO_2 values. A highly significant ($P<0.001$) effect of $pvCO_2$-risk on haematocrit values implies that HRc birds suffered from an insufficient oxygen supply to tissues.

Discussion

A previous study (Scheele *et al.,* 2003a) with two stocks of fast growing broiler chickens demonstrated a substantial variability in CO_2 concentrations in venous blood at 11 days of age between birds within stocks. Moreover, high $pvCO_2$ values detected at day 11 predicted high values for API values, which is a reliable indicator for ascites susceptibility, at 5 wk of age. In the present study, one aim was to confirm the results from the previous experiment. We found, apart from differences in ranges of venous $pvCO_2$ between stocks, a noticeable variability in $pvCO_2$ values at 12 days of age within all stocks, including LR and MK strains. These measurements should represent as much as possible the situation of broilers in poultry houses. Therefore, blood sampling procedures were executed quickly by taking a bird from its cage and sampling its blood without preceding measures for standardising effects of feed intake and physical activity. Part of the variability of determined $pvCO_2$ values therefore might have been due to transient fluctuations in production of CO_2, but repeated measurements in the same birds at different ages showed that the same birds repeatedly produced the same high and the same low values. As a result in all stocks differences in mean $pvCO_2$ values between high and low $pvCO_2$–risk groups were maintained at all ages. Effects of transient fluctuations therefore were not relevant to the aim of the study. Moreover, Buys *et al.* (1998) demonstrated that there is a genetic component to increased $pvCO_2$ values in broilers; they found that the progeny of ascites sensitive broiler lines (incubated under normal conditions) had higher pCO_2 levels in venous blood at 6 weeks of age than the progeny of ascites resistant lines.

In all stocks, mean $pvCO_2$ values of low $pvCO_2$-risk groups at day 12 were similar and comparable to values given by Powell (2000) for domestic fowl. Mean $pvCO_2$ values of HRc birds differed markedly between modern breeds and old native strains. A convincing effect of high $pvCO_2$ values on API values found in this experiment in modern lines, confirmed results obtained in a previous study (Scheele *et al.*, 2003a). Respecting all stocks we observed in this study a critical range of mean $pvCO_2$ values in high $pvCO_2$-risk groups from 45 to 50 mm Hg at 12 days of age. Above this range, HRc birds showed clearly higher API values at wk 5 than LRc birds. Below this range, $pvCO_2$ values of HRc groups caused no perceptibly elevated API values compared to LRc groups. API values of HRc groups of LR and MK stocks were below 20% and not any pathological sign on hearts was found. A total absence of ascitic signs within LR and MK strains (*Table 1*) corresponded with unchanged low API values during ageing from 12 days to 5 wk of age. Similar API values in all stocks at day 12 and unchanged API values during ageing in LR and MK chickens agree very well with results of Burton and Smith (1969) and Sillau *et al.*

(1980) showing that API values of healthy chickens did not correlate with body weight and remained constant from 2 to 6 wk of age. In modern breeds in our study, except for LRc groups in the BC population, both HRc and LRc groups demonstrated at wk 5 manifestly higher API values (25% and higher) than determined in complete flocks at day 12 and also significantly ($P<0.05$) higher values than found in LR and MK populations at wk 5. Increasing API values during the experimental period and API values of 25% and higher at 5 wk of age suggest a development of cardiovascular disorders. These results obtained also in LRc groups of sire and dam lines of modern breeds at 5 wk of age points at a damaged state of health of all pure broiler line chickens, used in our experiments.

Differences in selection traits between fast growing sire lines and slower growing dam lines were accompanied by distinct differences in ascites incidences and mortality. Sire lines that combined a fast growth with a low feed conversion ratio showed by far the most incidences of ascites. On the other hand, a very fast growing broiler cross (BC) was much less sensitive to ascites than a slower growing sire line (S2). Fast growing LRc BC birds showed at wk 5 similar API values than ascites resistant breeds LR and MK. The latter confirms results of Scheele *et al.* (2003a) showing constant low API values and no signs of ascites within fast growing LRc Ross chickens.

Within modern breeds, HRc groups of the slowest growing breed D2 showed at wk 5 extremely high API values. The combined results indicate that possible effects of selection traits on susceptibility ascites cannot be simply attributed to differences in growth rate. Other marked differences between sire and dam lines and the commercial hybrid may elucidate more causal relationships to ascites incidences. High mean $paCO_2$ (higher than 60 mm Hg) and low pH and paO_2 values in arterial blood of sire lines differed at wk 5 highly significant ($P< 0.001$) from values found in dam lines and in BC birds. Wideman *et al.* (1999) demonstrated that even a short period of 5 minutes of a modest increase in partial pressure of CO_2 (up to 55 mm Hg) together with a reduced pH in arterial blood of broilers was sufficient to trigger a significant increase in pulmonary arterial pressure, predisposing the birds to ascites. Reeves *et al.* (1990) discovered that at sea level, ascites susceptible chickens have impaired ventilatory drives and that they have relative hypoventilation at an early age. Especially high CO_2 concentrations in arterial blood might be originated by hypoventilation.

At all ages, differences in mean $pvCO_2$ values between high and low $pvCO_2$-risk groups in our study were similar for all modern lines and showed no relationship to growth rate. API values, as reliable indicators for ascites susceptibility, of modern breed chickens correlated with $pvCO_2$ values and not with growth rate. No differences in API values at wk 5 between HRc and LRc groups of D1 birds might be due to a combined effect of a relatively low mean $pvCO_2$ value in HRc groups at day 12 and fast increasing $pvCO_2$ values also in LRc groups of D1 during the experimental period. High $pvCO_2$ and $paCO_2$ values (50 mm Hg and higher) found in this experiment at wk 4 and 5 were comparable with CO_2 tensions in venous and arterial blood of ascites susceptible modern broiler populations kept at different ambient temperatures (Buys *et al.*, 1999; Korte *et al.*, 1999; Olkowski *et al.*, 1999; Scheele *et al.*, 2003a).

Generally, prominent differences in $pvCO_2$ values between $pvCO_2$-risk groups of modern broiler lines at day 12 were accompanied with smaller or no differences in pvO_2 values. Important differences in $pvCO_2$ values between high and low $pvCO_2$-risk groups of BC birds at day 12 and of S1 birds at day 21 predicting ascites susceptibility at wk 5 were not accompanied by statistically significant differences in pvO_2 values. Consequently, at an early age pvO_2 values were less predictive for high pulmonary pressure induced ascites at wk 5 than $pvCO_2$ values. As paO_2 values, contrary to $paCO_2$ values, were not significantly affected by $pvCO_2$-risk. This suggests that high carbon dioxide tensions in arterial blood were generated by a factor that influenced oxygen

tensions much less. All discussions of the adequacy of ventilation come directly from the alveolar ventilation equation wherein production and pCO_2 values are the only two explanatory variables ($paCO_2$ reciprocally related to alveolar ventilation). Consequently, abnormal values for $paCO_2$, at a given level of CO_2 production, first point at abnormal alveolar ventilation. Irregularities in blood gas tensions shown in this experiment started by signs of hypercapnia (normoxic hypercapnia in BC birds) whereas evident signs of hypoxemia were shown at the end of the experimental period. Low pvO_2 and paO_2 values at wk 4 and 5 in modern breeds (predominantly in sire lines) compared to ascites resistant lines may have contributed to pulmonary vasoconstriction and right ventricular hypertrophy in ascites susceptible high $pvCO_2$-risk birds.

Experimental results obtained in other species point at the important role of hypercapnia in activating vasoconstriction and high blood pressures. Studies with dogs revealed that hypercapnia not only increased pulmonary arterial pressure but also the central venous and systemic arterial pressure and decreased heart rate (Rothe *et al.*, 1985). Results of investigations by Anderson *et al.* (1996) with micro pigs suggest that hypercapnia and a decreased pH participate in blood pressure regulation via increased renal sodium/hydrogen exchange and renal sodium retention. Carbon dioxide induces a hypersecretion of norepinephrine (inducing vasoconstriction) by stimulating the sympathetic nervous system through a direct central action (Korner, 1979). Endothelin-1, a potent vasoconstrictor peptide produced by vascular endothelial cells, has direct and vigorous vasoconstrictor effects and enhances adrenergic vasoconstriction (Tabuchi *et al.*, 1989). Fontana *et al.* (2000) found strikingly higher levels of endothelin-1 and norepinephrine in arterial blood induced by high pCO_2values than induced by low pO_2 levels. Yanagisawa *et al.* (1988) showed a dose-response relationship of the potent vasoconstrictor effect of endothelin-1 on pulmonary artery branches in humans, whereas Martinez-Lemus *et al.* (2003) demonstrated the vasoconstriction responses to endothelin-1 in pulmonary artery rings from broiler chickens. The dominant effect of especially high pCO_2 values on the release of endothelin-1, as revealed by Fontana and co-workers, highlights the relationship between hypercapnia and increased pulmonary arterial pressure followed by right ventricular hypertrophy and ascites.

Haematocrit values, at day 12, in our study were significantly ($P< 0.001$) affected by both experimental factors. Mean haematocrit values of ascites susceptible sire lines were lower than found in ascites resistant breeds LR and MK, suggesting no relationship between haematocrit and ascites susceptibility at this age. Comparable to the effect of $pvCO_2$-risk on pO_2 the results showed that there was no consistent relationship between $pvCO_2$ and haematocrit values at day 12. These results are in agreement with findings that an increased susceptibility to ascites is not necessarily linked to high haematocrit values (Buys *et al.,* 1999; Scheele *et al.,* 2003b). After day 12, haematocrit values as indicators for hypoxemia increased steadily during ageing in modern breeds but not in ascites resistant breeds. Also differences in haematocrit values, between high and low $pvCO_2$-risk groups of modern breeds increased noticeably during ageing. High mean haematocrit values in venous blood at wk 4 and in arterial blood in wk 5 in breeds S1, S2 and D2 corresponded manifestly with high mean values for pulmonary arterial pressure index in these birds. Studies with humans at high altitudes showed that polycythemia in part was caused by a reduced ventilatory drive (Kryger *et al.* 1978). Particularly, as to the effects on haematocrit in our study, ascites susceptible ageing birds may undergo a delayed effect of a reduced respiratory activity (clearly inducing high $pvCO_2$ values) on availability of oxygen and subsequently on erythropoiesis and increasing haematocrit values.

Our results indicate that hypercapnia combined with low pH values and followed by hypoxemia (inducing high haematocrit values) provoked a marked high incidence of ascites and high API values in modern breeds.

Combined effects of different factors may reinforce as well a reduced oxygen delivery to tissues as pulmonary vasoconstriction. Due to "the Bohr effect" respiratory acidosis reduces the affinity of haemoglobin for oxygen, thus less oxygen is transported to the tissues due to a lower percentage saturation of haemoglobin with oxygen. Heart tissue anoxia may be involved in heart failure and hypertrophy. Data presented by Isaacks *et al.* (1986) indicate that increasing H^+ and CO_2 concentrations markedly affect the O_2 affinity of avian blood, particularly at pH near 7.4, which is close to the value found in our experiment. Rudolph and Yuan (1966) found in the new-born calf that the lower the pH the greater was the pulmonary vascular resistance response to pO_2 reduction.

Moreover, hypercapnic acidosis has been shown to produce negative myocardial inotropic effects in the dog (Noble *et al.*, 1967) and to elicit a compensatory baroreflex-mediated bradycardia in the conscious rat (Walker and Brizee, 1990). Korte *et al.* (1999) found a distinct effect of high $pvCO_2$ values in juvenile chickens on incidences of cardiac arrhythmias.

As to the present study: The increasing $pvCO_2$ values during ageing of all investigated groups of modern breeds including low risk groups, contrary to ascites resistant old breeds, may predict that eventually all birds of modern breeds, sooner or later, have a great risk of death from cardiac disorders or from ascites within a six week growth period. Although other factors than hypercapnia, such as hypothyroidism instigating a reduced mitochondrial function and an inefficient oxidative phosphorylation (Scheele *et al.,* 1992; 2003a), might also contribute to the occurrence of ascites; the present results suggest that high pCO_2 values are clearly related to ascites susceptibility. A recuperation of blood gas values at different ages in modern breeds to values as found in ascites resistant breeds is needed to reduce importantly the occurrence of the ascites syndrome at sea level. Not only because the economical losses, but also from the viewpoint of animal welfare; aspects of health should get more attention in genetic selection programs.

Balog (2003) emphasised that genetic selection is the best solution for eliminating the ascites syndrome in the near future. Our recommendation is that a strong genetic selection pressure on low $pvCO_2$ values at an early age might be a practical and effective action to prevent modern broiler populations for a further deteriorating state of health. Attention has to be paid to constant low $pvCO_2$ values during the whole growth period, comparable to the situation as found in ascites resistant old breeds. Such a selection might be accompanied by a selection for maximal growth rate, as long as there is no evidence that a fast growth is related to high CO_2 tensions in venous blood of chickens.

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Table 1 Final body weight (BW) at 33 days of age and feed conversion ratio (FCR) from 16 to 33 days of age in broilers from seven genetically different stocks[1]. Pulmonary arterial pressure index (API) values were measured at day 15 and incidences of the ascites syndrome[2] determined from 16 to 35 days of age in all dead birds including observations in sacrificed selected birds at 35 days of age (% of birds present at the start).

Stock	BW (g) 33 days	FCR (g/g) 16-33 days	API (%) 14 days	Ascites syndrome (%) 16 –35 days
S1	1790	1.69	20	19.2
S2	1661	1.71	21	27.8
D1	1486	1.78	21	3.8
D2	1455	1.80	22	5.8
BC	1736	1.74	20	7.7
LR	917	2.00	18	0.0
MK	921	2.19	18	0.0
P-value[3]	***	***	n.s.	***
LSD[4]	55	0.04	3	9.0

[1]Two pure sire lines (S1 and S2), two pure dam lines (D1 and D2), a commercially available broiler cross (BC) and two ascites resistant strains, native in France and Belgium (LR and MK).
[2]Observed signs of ascites, heart failure syndrome, and liver cirrhosis were classified as ascites syndrome
[3]*** P< 0.001; n.s.: not significant (P≥0.05)
[4]LSD = least significant difference

Table 2 Probability values[1] of significance of main and interactions effects of two experimental factors[2] on blood gas tensions ($pvCO_2$ and pvO_2) and on pH and haematocrit values in venous blood of broiler chickens at different ages and on pulmonary arterial pressure index (API) values determined at 35 days of age.

Response parameter	Age (days)	Stock (S)	$pvCO_2$-risk (Rc)	S x Rc interaction
$pvCO_2$ (mm Hg)	12	***	***	***
	21	***	***	**
	28	***	***	**
pvO_2 (mm Hg)	12	***	***	*
	21	***	***	*
	28	***	***	***
pH	12	***	***	-
	21	***	***	-
	28	***	***	-
Haematocrit (%)	12	***	***	-
	21	***	***	*
	28	***	***	-
API (%)	35	***	***	***

[1]*: P< 0.05; **: P<0.01; ***: P<0.001; -: not significant (P≥0.05)
[2]Two factors; 1) Stock (S) = seven different genetic groups of broiler chickens, two pure sire lines (S1 and S2), two pure dam lines (D1 and D2), a commercially available broiler cross (BC) and two ascites resistant strains, native in France and Belgium (LR and MK). 2) $pvCO_2$-risk (Rc) = two groups of birds within each stock selected on high and low $pvCO_2$ (HRc and LRc) in venous blood at 12 days of age

Table 3 Measurements in high pCO_2-risk (HRc) and low pCO_2-risk (LRc) groups of birds per stock[1]. Mean values for pH at day 12, for $pvCO_2$ at different ages in venous blood of chickens, and for pulmonary arterial pressure index (API) at 35 days of age.

Experimental factors		Mean values at different ages				
Stock	$pvCO_2$-risk	pH	$pvCO_2$ (mm Hg)			API %
		12 days	12 days	21 days	28 days	35 days
S1	HRc	7.36	52.4	57.1	59.0	31
	LRc	7.43	36.1	47.7	49.0	26
S2	HRc	7.36	51.7	59.5	63.6	32
	LRc	7.42	39.8	47.9	49.1	27
D1	HRc	7.38	47.9	56.0	59.9	25
	LRc	7.43	37.6	45.4	48.6	25
D2	HRc	7.36	53.1	57.3	63.1	37
	LRc	7.43	40.3	45.4	50.7	24
BC	HRc	7.34	50.4	58.4	59.2	26
	LRc	7.43	37.7	44.3	47.6	23
LR	HRc	7.39	42.6	45.3	45.6	19
	LRc	7.45	35.7	40.1	39.8	20
MK	HRc	7.35	45.2	42.9	44.8	18
	LRc	7.45	35.6	38.9	39.9	18
LSD[2]		0.02	1.8	3.8	4.1	4

[1]Two experimental factors; 1) Stock (S) = seven different genetic groups of broiler chickens, two pure sire lines (S1 and S2), two pure dam lines (D1 and D2), a commercially available broiler cross (BC) and two ascites resistant strains, native in France and Belgium (LR and MK). 2) $pvCO_2$-risk (Rc) = two groups of birds within each stock selected on high and low $pvCO_2$ (HRc and LRc) in venous blood at 12 days of age.
[2]LSD = least significant difference.

Table 4 Measurements in high pCO_2-risk (HRc) and low pCO_2-risk (LRc) groups of birds per stock[1]. Mean values for pvO_2 and haematocrit values in venous blood of chickens at different ages.

Experimental factors		Mean values at different ages				
Stock	$pvCO_2$-risk	pvO_2 (mm Hg)			Haematocrit(%)	
		12 days	21 days	28 days	12 days	21 days
S1	HRc	41.0	39.8	34.7	29.8	33.2
	LRc	48.3	43.7	40.7	27.8	31.0
S2	HRc	43.6	35.6	29.4	29.0	32.9
	LRc	47.3	44.2	40.7	27.7	31.2
D1	HRc	47.0	43.0	41.1	30.0	32.6
	LRc	51.3	47.9	44.9	30.0	30.9
D2	HRc	43.9	40.7	35.1	33.3	36.7
	LRc	48.4	46.4	44.9	31.5	33.1
BC	HRc	48.3	39.7	37.4	29.0	33.0
	LRc	48.8	44.6	41.9	28.9	31.3
LR	HRc	51.7	50.2	48.1	30.2	31.0
	LRc	50.9	49.8	50.6	30.2	30.3
MK	HRc	51.1	55.4	52.4	29.1	29.5
	LRc	50.3	52.9	50.7	29.2	30.2
LSD[2]		3.8	4.6	4.3	1.4	1.6

[1]Two experimental factors; 1) Stock (S) = seven different genetic groups of broiler chickens, two pure sire lines (S1 and S2), two pure dam lines (D1 and D2), a commercially available broiler cross (BC) and two ascites resistant strains, native in France and Belgium (LR and MK). 2) $pvCO_2$-risk (Rc) = two groups of birds within each stock selected on high and low $pvCO_2$ (HRc and LRc) in venous blood at 12 days of age.
[2]LSD = least significant difference

Table 5 Mean values per experimental factor[1] for pH, and haematocrit in venous blood of broiler chickens at different ages.

Age	Stock								pCO_2 risk		
(days)	S1	S2	D1	D2	BC	LR	MK	LSD[2]	HRc	LRc	LSD[2]
pH											
21	7.36	7.35	7.37	7.38	7.36	7.40	7.42	0.02	7.35	7.41	0.01
28	7.36	7.34	7.34	7.35	7.35	7.38	7.39	0.02	7.32	7.39	0.01
Haematocrit (%)											
28	32.3	32.9	30.6	33.4	32.4	29.2	29.2	1.8	32.2	30.6	0.7

[1]Two factors; 1) Stock (S) = seven different genetic groups of broiler chickens, two pure sire lines (S1 and S2), two pure dam lines (D1 and D2), a commercially available broiler cross (BC) and two ascites resistant old strains, native in France and Belgium (LR and MK). 2) $pvCO_2$ risk (Rc) = two groups of birds within each stock selected on high and low $pvCO_2$ (HRc and LRc) in venous blood at 12 days of age

Table 6 Probability values[1] of significance of main and interaction effects of two experimental factors[2]: on $paCO_2$, paO_2, pH and haematocrit in arterial blood of broiler chickens at 5 wk of age.

Response variable	Stock (S)	$pvCO_2$ risk (Rc)	S x Rc interaction effect
$paCO_2$ (mm Hg)	***	***	-
paO_2 (mm Hg)	***	-	-
pH	***	-	-
Haematocrit (%)	***	***	**

[1]***: P<0.001; -: not significant (P≥0.05)
[2]Two factors; 1) Stock (S) = seven different genetic groups of broiler chickens, two pure sire lines (S1 and S2), two pure dam lines (D1 and D2), a commercially available broiler cross (BC) and two ascites resistant old strains, native in France and Belgium (LR and MK). 2) $pvCO_2$ risk (Rc) = two groups of birds within each stock selected on high and low $pvCO_2$ (HRc and LRc) in venous blood at 12 days of age

Table 7 Mean values per experimental factor[1] for $paCO_2$, paO_2, pH, and haematocrit (Ht) and the interaction effect of the two experimental factors on Ht in arterial blood of broiler chickens at 5 wk of age.

Variable	Stock								pv CO_2 risk		
	S1	S2	D1	D2	BC	LR	MK	LSD2	HRc	LRc	LSD[2]
$paCO_2$ (mm Hg)	60.4	60.3	47.9	53.0	52.2	43.9	44.1	3.4	53.1	50.3	1.6
paO_2 (mm Hg)	35.7	38.3	48.6	41.8	42.1	51.0	50.2	4.4	43.4	44.5	2.1
pH	7.33	7.31	7.39	7.38	7.36	7.36	7.38	0.02	7.36	7.36	0.01
Ht (%)	35.3	36.1	30.9	34.2	34.0	29.5	29.9	1.8	33.8	31.9	0.8
Ht (%) HRc	37.0	38.9	32.1	35.7	33.7	29.1	29.4	2.6			
Ht (%) LRc	33.4	33.1	29.4	32.8	33.0	29.6	30.1				

[1]Two factors; 1) Stock (S) = seven different genetic groups of broiler chickens, two pure sire lines (S1 and S2), two pure dam lines (D1 and D2), a commercially available broiler cross (BC) and two ascites resistant old strains, native in France and Belgium (LR and MK). 2) $pvCO_2$ risk (Rc) = two groups of birds within each stock selected on high and low $pvCO_2$ (HRc and LRc) in venous blood at 12 days of age.